



# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>L80001215WO</b>	<b>FOR FURTHER ACTION</b>		See Form PCT/PEA/416
International application No. <b>PCT/CA2004/000606</b>	International filing date (day/month/year) <b>22.04.2004</b>	Priority date (day/month/year) <b>22.04.2003</b>	
International Patent Classification (IPC) or national classification and IPC <b>A01N43/16, A01N63/02</b>			
Applicant <b>BIOS AGRICULTURE INC. et al.</b>			
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p style="margin-left: 20px;">a. <input checked="" type="checkbox"/> sent to the applicant and to the International Bureau a total of 7 sheets, as follows:</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>			
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>			
Date of submission of the demand  <b>22.02.2005</b>		Date of completion of this report  <b>25.07.2005</b>	
Name and mailing address of the international preliminary examining authority:   European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465		Authorized Officer  <b>Klaver, J</b>  Telephone No. +49 89 2399-8601  	

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**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**International application No.  
PCT/CA2004/000606**Box No. 1 Basis of the report**

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language, which is the language of a translation furnished for the purposes of:

- ☐ international search (under Rules 12.3 and 23.1(b))  
☐ publication of the international application (under Rule 12.4)  
☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the elements\* of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

**Description, Pages**

6-25 as originally filed  
 1-5, 5a received on 03.03.2005 with letter of 22.02.2005

**Claims, Numbers**

1-6 received on 03.03.2005 with letter of 22.02.2005

**Drawings, Sheets**

1-15 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages  
☐ the claims, Nos.  
☐ the drawings, sheets/figs  
☐ the sequence listing *(specify)*:  
☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages  
☐ the claims, Nos.  
☐ the drawings, sheets/figs  
☐ the sequence listing *(specify)*:  
☐ any table(s) related to sequence listing *(specify)*:

\* If item 4 applies, some or all of these sheets may be marked "superseded."

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**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability;  
citations and explanations supporting such statement**

1). The subject-matter of claims 1 - 6 does not meet the criteria of Art. 33 (2) PCT since it is not novel with respect to the prior art:

- Agriculturally acceptable compositions comprising an effective amount of lipochitooligosaccharides (LCO's) have been disclosed in all documents cited in the International Search Report.

It is pointed out, that an intended purpose does not render a composition novel with respect to a composition essentially containing the same ingredients. It is evident from, e.g., Prithiviraj et al., 2003 (= D1), Atti et al., 2002 (= D2) and WO 01/26465 A1 (= D3), that the concentrations of LCO in the compositions of the prior art are in the same ranges as used in the present application. The subject-matter of composition claim 6 hence is not novel.

- D1 discloses on page 440, last paragraph: "In general, LCO-treated plants developed faster, flowered earlier and had more flowers than the controls (results not shown). At 15 days after seeding, plants treated with the various LCOs showed 40-100% bolting as compared to 20-14% in the controls."

This statement anticipates the use and method as defined by present claims 1 - 5: Claims 1 and 2 define the use of LCO or an LCO comprising composition for acceleration of flowering in a plant, claims 3 and 4 define the use of LCO or an LCO comprising composition for acceleration of flowering and fruiting or for increasing flower numbers and associated yields, whereas claim 5 defines a method for acceleration of flowering and/or fruiting by application of an LCO comprising composition to a plant.

None of these claims contain a feature which distinguishes its claimed subject-matter from these statements in D1, which obviously are based on performed, albeit unpublished, experiments.

Applicant essentially argues in his letter of 22.02.05, that this is a 'bald statement' not supported by any experimental evidence. In his view, it would not be possible to correlate

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conclusions drawn in D1 with the experimental results.

This argument cannot be followed:

The baldness of a statement is not relevant for the plausibility of its contents or for the message it carries.

D1 itself discloses, that the conclusion regarding the observed accelerated and enhanced flowering are not based on the data as given in the publication itself but are based on unpublished results. This does not make the statement itself irrelevant or implausible, especially since this observation is fully in line with the results of the published experimental data which relate to increased emergence and improved germination. It thus may be considered a further illustration of the advantageous effects to be expected from application of LCO's to plants.

It is evident from D1, that before the priority date of the present application LCO's have been applied to plants and that this use resulted in an accelerated flowering and in increased flower yields. It furthermore does not require any special technical skills to execute the experiments mentioned in this particular passage of D1.

The subject-matter of claims 1 - 5 hence has been made available to the public by D1 and thus is not novel (Art. 33 (2) PCT).

- D2 discloses, that LCO treatment of soybeans under medium water stress conditions results in an increase of flower induction, pod development and plant height, which effects are not clearly and unequivocally distinguished from the terms 'acceleration of flowering' and 'Increasing flower numbers and/or associated yield' as used in present claims 1 - 5.

Applicant argues (letter of 22.02.05), that the positive effects of LCO only have been demonstrated in one of three experiments under water stress conditions and that these experiments do not give any indication on effects under normal growth conditions. The latter statement is not relevant, since the present claims are not restricted to normal growth conditions. It is even derivable from the description that such conditions are envisaged by the present claims ((amended) page 5, last paragraph).

In the absence of the full text of D2, it furthermore cannot be established what the precise content of this disclosure is. On the basis of the published abstract, however, one can only conclude, that it was known in the art, that application of LCO to soybeans under medium water stress conditions resulted in the same effects as encompassed by the use and methods as defined by present claims 1 - 5. Said claims hence are not novel with respect to D2.

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- D3 discloses on page 19, line 1 - 7, that leaf application of LCO to plants results in more leaf area and dry weight and, in the case of soybeans, resulted in increased branch number, leaf area, pod number, plant dry matter and grain yield. It is further *expressis verbis* disclosed in D3, that application of LCO will result in an increased productivity in a wide range of crops (D3: page 19, line 6/7). Example 5 furthermore discloses, that LCO treatment of soybeans resulted in increased growth and an increased number of pod clusters per plant, number of pods and total number of seeds per plant (D3: page 27, last paragraph).

The effects as mentioned in D3 are within the scope of the uses and methods as defined by present claims 3 - 5, as can be seen from (amended) page 5, last paragraph, of the present description where it is stated: "The uses (...) and methods of the present invention (...) include initiation of early bud and/or flowering and/or increased flowering (...), leading to earlier fruit development and/or enhanced plant maturity and/or plant growth and yield (...)".

D3 hence anticipates the novelty of claims 3 - 5, at least insofar as these claims define the use of LCO for increasing flower numbers and associated yield. The fact, that the examples of D3 do not specifically disclose the exact change in the numbers of flowers is not relevant in view of the disclosures of D3 as cited here above.

The presently claimed uses, methods and compositions as defined by claims 1 - 6 are not novel (Art. 33 (2) PCT).

2). Even if the subject-matter of the claims would be considered novel, it cannot be seen which particular technical problem has been solved by the present application which was not already solved by the prior art. It is obvious for the skilled artisan trying to find further advantageous applications for LCO's to test the observations made in D1 (page 440, last paragraph) on the list of plants as disclosed in D3. Such a test would inevitably result in the claimed uses and methods as defined by present claims 1 - 5.

Claims 1 - 5, even if considered novel, hence are not considered to be based on an inventive step and do not meet the criteria set forth in Art. 33 (3) PCT.

3). The industrial applicability is evident (Art. 33 (3) PCT).

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**USE OF LIPO-CHITOLIGOSACCHARIDES TO INITIATE EARLY FLOWERING  
LEADING TO INCREASED FLOWER NUMBERS AND INCREASED YIELD IN  
PLANTS AND RELATED METHODS AND COMPOSITIONS**

**Field of the Invention**

The present invention relates generally to the fields of agriculture and horticulture, including but not limited to agricultural crops, flowers, fruits, vegetables, nuts, turfgrass, herbs, spices, ornamental shrubs and trees, aquatic plants, tubers, minitubers, microtubers, corms and mushrooms grown outdoors or in greenhouses or indoors for both commercial or personal use and agriculture and more specifically to the use of Lipo-chitooligosaccharides (LCOs) and compositions thereof to induce early flowering, increase the number of buds and flowers, initiate earlier fruiting, earlier maturity and increase yields in plants and to methods of inducing earlier flowering and initiation of earlier fruiting in plants by exposure to LCOs and compositions of same.

**Background of the Invention**

There is a growing interest in the role of LCOs and compositions thereof for enhancement of plant seed germination, seedling emergence and growth of plants both for crop and horticultural purposes in both legumes and non-legumes. Compositions for accelerating seed germination and plant growth are provided in Application No. PCT/CA99/00666, published February 3, 2000, WO 00/04778, all contents of which are incorporated herein by reference. There is also an interest in the possible effects of LCOs in plant photosynthesis and PCT/CA00/01192, published April 19, 2001, WO 01/26485 A1 describes the use of LCOs and compositions of LCOs for increasing plant photosynthesis. Chemical structures of LCOs are described in U.S. Patent Nos. 5,175,149; 5,321,011 and 5,549,718. Synthetic LCOs are also known.

There is great interest in the field of agricultural research, particularly in the field of plant growth promoters, of plant physiological processes which may be affected by LCOs. Prithiviraj et al, Planta (2003) 216:437-445, "A host-specific bacteria-to-plant signal molecule (Nod factor) enhances germination and early growth of diverse crop

plants" discuss certain observed induced physiological changes in both host and non-host plants by LCOs, all contents of which is incorporated herein by reference. Atti et al, conference paper ICID Young Professionals Forum, 18<sup>th</sup> International Congress on Irrigation and Drainage, Montreal, Canada, 2002, pages 1 to 11, discuss certain observed physiological changes when LCOs are administered under drought-stress conditions.

WO 01/26465 A1 is directed to use of LCOs in association with increased stomatal conductance and enhanced photosynthetic activity. LCOs are known to be released by *Rhizobia*, symbiotic bacteria primarily of the genera *Generarhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium* and *Azorhizobium* and the like, the Rhizobiaceae family being in a state of taxonomic flux. Both of the aforesaid International applications summarize current understanding of the specialized symbiotic relationship of *Rhizobia* with legume host plants in the formation of nodule organs and associated fixation of atmospheric nitrogen within these organs, as well as the plant to bacteria signal and bacteria to plant signal interaction associated with such symbiotic relationship.

Although there is a considerable body of knowledge on the influence of LCOs, including synthetic LCOs, on typical host plant physiology, there is a growing interest in the effect of LCOs on plant growth with respect to both host and non-host plants, particularly by application of the molecule without necessarily the fostering of a micro-organism and plants symbiotic relationship.

The body of knowledge relating to the possible role of LCOs, including synthetic LCOs, in both host and non-host plants and on processes associated with plant growth promotion continues to grow, with particular practical interest in the effects of LCOs on plant physiology and processes relevant to increase plant yields, not only with respect to commonly considered crop plants, including agricultural crops, both host and non-host, but also with respect to horticulture species.

Thus, there continues to be a need to study the effects of LCOs, including synthetic LCOs, on plant growth, in addition to processes relating to nodulation and nitrogen fixation in legume host plants and to germination, emergence and photostimulation in

both legume and non-legume plants. In particular, there is a need to study the effect of LCOs on bud and flowering initiation, budding, fruiting initiation and development, generally in relation to growth and maturity of plants, both leguminous and non-leguminous and the affect on plant yields, all in contrast and in addition to the effects as described in the above-cited references. The present invention endeavours to address these and other needs.

#### Summary of the Invention

The present invention relates to the use of LCOs in initiating early flowering and budding, increased flowering and budding, leading to earlier fruit development in non-legume and legume plants, as compared to flowering and fruit development under conditions without use of LCOs, and the enhancement of plant growth and yield associated therewith. The present invention also relates to agricultural compositions comprising an effective amount of at least one LCO and agriculturally acceptable carriers, associated with early flowering and budding, increased flowering and budding, earlier plant maturity and earlier initiation of fruit development as compared to conditions without use of LCOs, and with increased growth and plant yield. The present invention further relates to methods using LCOs and compositions of one or more LCOs and agriculturally acceptable carriers, associated with earlier flowering initiation and budding, increased flowering and budding and earlier plant maturity leading to initiation of earlier fruit development in both legume and non-legume crop plants as compared to conditions without use of LCOs and otherwise associated enhancement of growth and yield, and all as exemplified herein below.

Surprisingly, the compositions of the present invention affect not only legume varieties but also a wide and divergent variety of non-legume plants, including crop plants and horticultural and bedding plant species in the initiation of earlier flowering and budding, increased flowering and budding, earlier maturity and earlier fruit development, and increased yield, as compared to conditions where LCOs are not applied and all as exemplified herein below.

According to the present invention, in both legume and non-legume plants, the administration of an effective amount of LCO or LCOs, or of compositions of one or more LCOs with agriculturally suitable carriers, initiates budding and/or flowering at an earlier stage, increases total bud and/or flower numbers and leading to earlier fruit



development and plant maturity as compared to conditions without use of LCOs, including an associated increase in yield. Administration of LCOs for such purpose may be by leaf or stem application, or application in the proximity of the seed, root or plant. Such methods are non-limiting and may include other methods, which would be understood by the skilled person, including by administration of micro-organisms known to release LCOs in the proximity of a plant seed, tuber, corn, or seedling in any stage of emergence, or in the proximity of a plant, including in the vicinity of the root and root hairs. The same would be with respect to application of LCOs independent of the micro-organisms known to release such molecules.

Thus, in accordance with a further embodiment of the present invention, there is provided a method for the initiation of earlier flowering, increased budding and flowering, leading to earlier fruit development and plant maturity in non-legume and legume plants associated with the growth and yield of a plant, comprising the treatment of a plant with an effective amount of one or more LCOs or a composition comprising an agriculturally effective amount of one or more LCOs in association with an agriculturally suitable carrier or carriers, wherein the effective amount has the effect of initiating earlier flowering and/or budding and/or increased bud and/or flower number, leading to earlier fruit development and/or plant growth and/or yield, as compared to an untreated plant, and all as exemplified herein below. Suitable LCOs for use according to the present invention include the LCOs as identified in the aforesaid international applications and patents.

Compositions of the present invention will be understood to include in their scope, one or more different LCO molecules, including synthetic molecules, as well as comprising one or more types of molecules other than LCO, including, without limitation, one or more plant to bacteria molecule and/or other molecules or agents known to promote growth or fitness and mixtures of such compositions.

The inventors and applicant herein are the first to show, as exemplified in the greenhouse and field experiments set out hereafter, that a composition comprising an LCO can have a significant affect on both legume and non-legume plants by initiating early bud and/or flowering, increased bud and/or flowering, leading to earlier fruit development and/or yield, as compared to conditions without use of LCOs, and the enhancement of plant maturity, growth and yield associated therewith. Non-limiting examples of crop plants include dicotyledons and monocotyledons and

legumes. From the aforesaid experiments and as set out below, it can be predicted that such results will apply to crop, including agricultural crop, horticultural and personal use plants, legumes and non-legumes, including, but not limited to, flowers, fruits, vegetables, nuts, tubers, turf grass, herbs, spices, ornamental shrubs and trees, aquatic plants and mushrooms grown in field or greenhouse for agricultural, commercial and personal use. In view of the plants exemplified herein and the results, the skilled person will appreciate, can adapt the teaching of the present invention to a diversity of plants, both legume and non-legume, for crop, horticultural and personal use, including but not limited to, plants of the families: Fabaceae, Brassicaceae, Solonaceae, Chenopodiaceae, Asteraceae, Malvaceae, Cucurbitaceae and Poaceae.

The term "LCO" as used herein, will be understood as reference in general to a Nod factor which is under control of at least one modulation gene common to rhizobia, that is bacterial strains which are involved in a nitrogen fixing symbiotic relationship with a legume, and which serve as micro-organism-to-plant phytohormones which induce the formation of nodules in legumes and enable the symbiotic micro-organisms to colonize said plant modules. LCOs, including synthetic LCOs are understood to comprise derivatives of an oligosaccharide moiety, including fatty acid condensed at one end thereof. Non-limiting examples of LCOs are described in U.S. Patent numbers 5,175,149; 5,321,011 and 5,549,718. The instant invention is demonstrated in particular with LCOs from *Bradyrhizobium japonicum*, but it not so limited.

The uses, compositions and methods of the present invention will be understood to include initiation of early bud and/or flowering and/or increased flowering and/or budding, leading to earlier fruit development and/or enhanced plant maturity and/or plant growth and yield under both sub optimal or limiting and non-limiting environmental conditions associated therewith. Such sub optimal or limiting environmental conditions include but are not limited to limiting or sub optimal conditions of heat, water pH, soil nitrogen concentrations and the like.

An effective amount of LCO will be understood to relate to uses, compositions and methods of the present invention wherein the amount is sufficient to manifest statistically significant earlier budding and/or flowering and/or increased flowering and/or budding, leading to earlier fruit development and/or enhanced maturity and/or plant growth and yield associated therewith.

By proximity of seed, tuber, corn, root or plant will be understood to relate to any location of seed, root or plant wherein soluble materials or compositions of the present invention will be in actual contact with said seed, root or plant.

By bud or budding will be understood conditions consistent with stem swelling consisting of overlapping immature leaves or petals. By flowering will be understood the process or state of producing one or more flower.

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**CLAIMS:**

1. Use of an agriculturally acceptable composition for acceleration of flowering in a plant comprising an effective amount of at least one Lipo-chitooligosaccharides with one or more agriculturally acceptable carrier.
2. Use of an agriculturally effective amount of Lipo-chitooligosaccharides for acceleration of flowering in a plant.
3. Use of an agriculturally acceptable composition comprising an effective amount of one or more Lipo-chitooligosaccharides, with one or more agriculturally acceptable carrier, for acceleration of flowering and fruiting in a plant or for increasing flower numbers and associated yield.
4. Use of an effective amount of one or more Lipo-chitooligosaccharides for acceleration of flowering and fruiting in a plant, or increasing flower numbers and associated yields.
5. A method for acceleration of flowering and/or fruiting in a plant, comprising the application to a plant of a composition comprising an effective amount of one or more Lipo-chitooligosaccharides with one or more agriculturally acceptable carrier, said amount effective to accelerate flowering or fruiting in a plant, or increasing flower numbers or associated yield as compared to conditions without treatment with Lipo-chitooligosaccharides.
6. An agriculturally acceptable composition for acceleration of flowering in a plant comprising an effective amount of at least one Lipo-chitooligosaccharides with one or more agriculturally acceptable carrier.